

# Complete genome sequence of *Dyadobacter fermentans* type strain (NS114<sup>T</sup>)

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## Keywords

mesophile, free-living, non-pathogenic, aerobic, chains of rods, *Cytophagaceae*

## Abstract

*Dyadobacter fermentans* (Chelius MK and Triplett EW, 2000) is the type species of the genus *Dyadobacter*. It is of phylogenetic interest because of its location in the *Cytophagaceae*, a very diverse family within the order ‘*Sphingobacteriales*’. *D. fermentans* has a mainly respiratory metabolism, stains Gram-negative, is non-motile and oxidase and catalase positive. It is characterized by the production of cell filaments in ageing cultures, a flexirubin-like pigment and its ability to ferment glucose, which is almost unique in the aerobically living members of this taxonomically difficult family. Here we describe the features of this organism, together with the complete genome sequence, and annotation. This is the first complete genome sequence of the ‘sphingobacterial’ genus *Dyadobacter*, and this 6,967,790 bp long single replicon genome with its 5804 protein-coding and 50 RNA genes is part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

## Introduction

*Dyadobacter fermentans* strain NS114<sup>T</sup> (DSM 18053 = ATCC 700827 = CIP 107007) is the type strain of the species, which is the type species of the genus *Dyadobacter*. *D. fermentans* was described by Chelius and Triplett in 2000 [1] as mainly aerobic, but also able to grow by fermentation of glucose, Gram-negative and nonmotile. The organism is of significant interest

for its position in the tree of life, because the genus *Dyadobacter* (currently 7 species, Figure 1) is rather isolated within the family *Cytophagaceae* [2], as it has less than 88% similarity of the 16S rRNA gene sequence to any other bacteria with standing in nomenclature, with *Persicitalea* and *Runella* as closest neighboring genera.

Strain NS114<sup>T</sup> was isolated in a study on the endophytic community of maize plants, where bacteria were isolated from plants which had been grown using surface sterilized seeds in autoclaved synthetic soil in greenhouses [1]. Plant stems were surface sterilized and crushed prior to plating. The organism was found in stem tissues of plants which were cultivated on a nitrogen-free nutrient solution, but not in the nitrogen-fertilized counterparts. The type strain of *Runella zeae* (NS12<sup>T</sup>) was co-isolated from the same material, although no microscopic evidence has been presented to date that members of these species were living within the plant tissue [1].

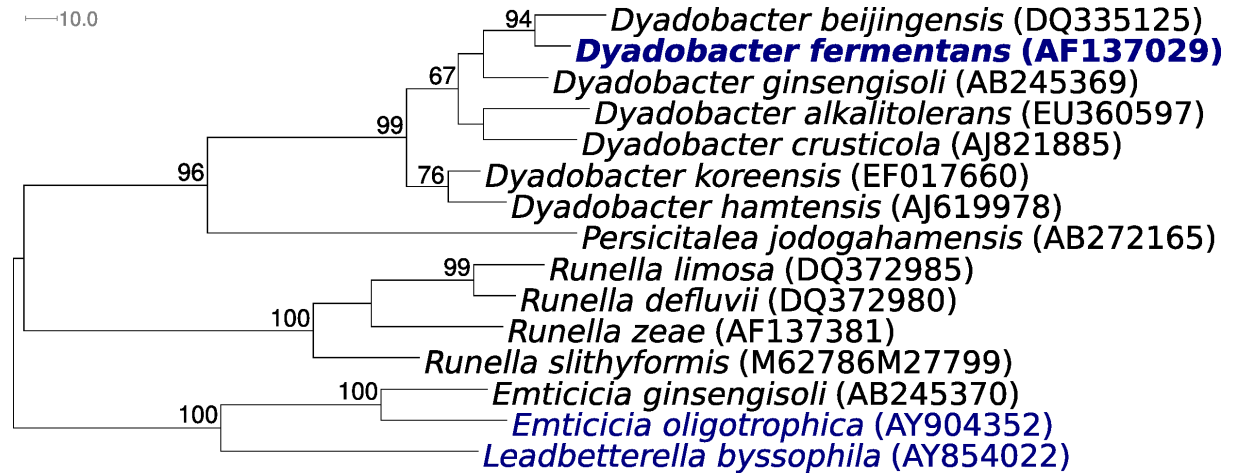
Members of the species *D. fermentans* were regularly found in cysts of the soybean nematode *Heterodera glycines* [3]. *D. fermentans* was also isolated from contaminated soil as a result of its ability to grow on 7,8-benzoquinoline [4], an N-containing heterocyclic aromatic hydrocarbon (azaarene) widely distributed in products of incomplete combustion processes, with toxic and cancerogenic effects. Two isolates from a lime stone cavern in Arizona (DQ207364 and DQ207362) display a significant 16S rRNA gene sequence similarity of 98%. Only few closely related phylotypes from environmental samples and global surveys are recorded to be highly related to *D. fermentans*, a fact suggesting that members of this taxon are not very abundant: Three clones of uncultured bacteria of a deep sea sediment collected at the Western Tropical Pacific Warm Pool in the Pacific Ocean (AM085468, AM085473 and AM085489) and one of the activated sludge of a membrane bioreactor (EU283373) are recorded (status May 2009). Intrageneric similarity within the genus *Dyadobacter* is rather low [5].

*D. fermentans* has been recognized by its flexirubin-like yellow pigment and by its growth as flocculent filaments of ovoid rods in old cultures [1]. The rod-shaped cells of strain NS114<sup>T</sup> occur polarly attached in groups of 2-4 cells during logarithmic growth, cells being frequently arranged at an angle to give V-formations. As the culture ages, irregular filaments or ovoid rods form (Figure 2) which sediment as fluffs in liquid culture [1]. The cells are Gram-negative, non-motile and non-sporulating, oxidase and catalase positive. Like many plant associated bacteria, strain NS114<sup>T</sup> produces copious amounts of slime when grown on nitrogen-limited agar. The organism grows aerobically, however is also reported to be able to ferment glucose and sucrose in the O/F-test in Hugh-Leifson's medium [1, 5], which according to our own observations might be an experimental artefact due to prolonged incubation [IDA]. The colony colour of strain NS114<sup>T</sup> is yellow to orange [1, 5]. The absorbance maximum of an ethanol extract of the cells is at 450 nm, and the absorbance peak broadens under alkaline conditions, which is a typical feature of flexirubin-like pigments [1]. This pigment was found in all six species of the genus *Dyadobacter* thus confirming that the presence of the pigment is a property of the genus [6-10].

Here we present a summary classification and a set of features for *D. fermentans*, strain NS114<sup>T</sup> (Table 1), together with the description of the complete genomic sequencing and annotation.

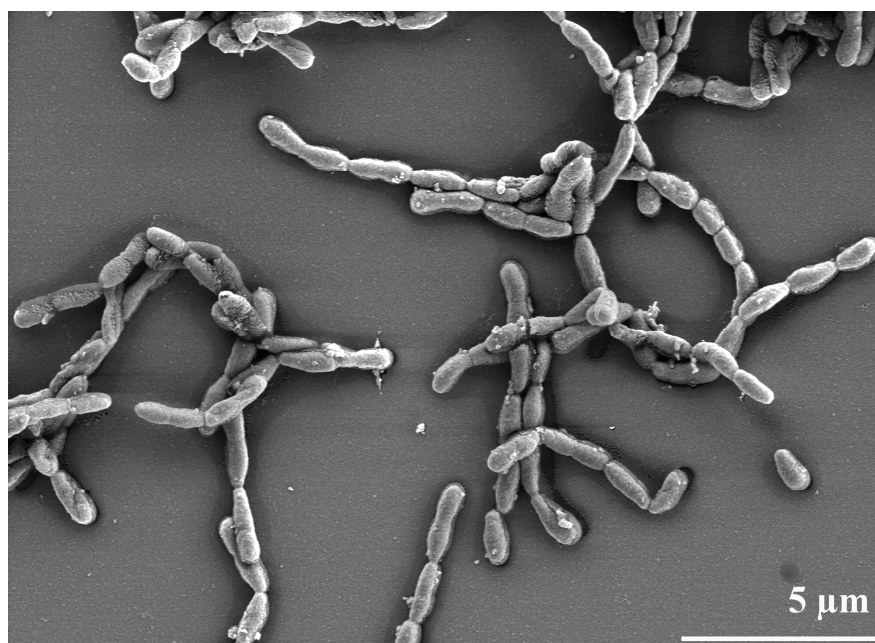
## Classification and features of organism

Figure 1 shows the phylogenetic neighborhood of *D. fermentans* strain NS114<sup>T</sup> in a 16S rRNA based tree. Analysis of the four 16S rRNA gene sequences in the genome of strain NS114<sup>T</sup> indicated that three copies are almost identical, and one differs by seven nucleotides from these. The sequences of the three identical 16S rRNA genes differ by two nucleotides from the previously published 16S rRNA sequence generated from DSM 18053 (AF137029). The slight differences between the genome data and the reported 16S rRNA gene sequence is probably due to sequencing errors in the previously reported sequence data.



**Figure 1.** Phylogenetic tree of *D. fermentans* strain NS114<sup>T</sup>, all type strains of the genus *Dyadobacter* and the most closely related *Cytophagaceae* type strains, inferred from 1378 aligned characters [11, 12] of the 16S rRNA sequence under the maximum parsimony criterion [13]. The tree was rooted with *Emticicia* and *Leadbetterella*, members of the family *Cytophagaceae*. The branches are scaled in terms of the minimal number of substitutions across all sites. Numbers above branches are support values from 1000 bootstrap replicates if larger than 60%. Strains with a genome sequencing project registered in GOLD [14] are printed in blue; published genomes in bold.

**Figure 2.** Scanning electron micrograph of *D. fermentans* NS114<sup>T</sup>



**Chemotaxonomy.** The whole cells fatty acid pattern of strain NS114<sup>T</sup> is dominated by unsaturated, and saturated iso-branched, straight chain unsaturated and large amounts of iso-branched, hydroxylated species. Major components are iso-C<sub>15:0</sub>-2OH and/or C<sub>16:1</sub>ω7c (43.5%), C<sub>16:1</sub>ω5c (17.5%) and iso-C<sub>15:0</sub> (16.8). Considerable amounts of the 3-hydroxylated fatty acids iso-C<sub>15:0</sub>-3OH, C<sub>16:0</sub>-3OH and iso-C<sub>17:0</sub>-3OH are detected [1]. The quinone composition has not been investigated for strain NS114<sup>T</sup>. The main component reported for the closely related type strains of *D. ginsengisoli* and *D. alkalitolerans* is menaquinone MK-7 [8, 10]. Cells of strain NS114<sup>T</sup> contain spermidine as the major cellular polyamine and putrescine, cadaverine and spermine as minor components. The latter compound was not detected in any of the three other strains studied to date of the family *Cytophagaceae*, representing *Flexibacter flexilis*, *Microscilla marina*, and *D. beijingensis* [15]. The polar lipid composition has not been investigated in either this strain or other members of the genus *Dyadobacter*.

**Table 1.** Classification and general features of *D. fermentans* NS114<sup>T</sup> in accordance with the MIGS recommendations [16]

MIGS ID	Property	Term	Evidence code <sup>a</sup>
MIGS-22	Current classification	Domain <i>Bacteria</i>	
		Phylum ' <i>Bacteroidetes</i> '	
		Class ' <i>Sphingobacteria</i> '	TAS [2]
		Order ' <i>Sphingobacteriales</i> '	TAS [2]
		Family <i>Cytophagaceae</i>	TAS [17]
		Genus <i>Dyadobacter</i>	TAS [1]
		Species <i>Dyadobacter fermentans</i>	TAS [1]
	Gram stain	Type strain NS114	
		negative	TAS [1]
		Cell shape	rods in pairs or chains
		Motility	nonmotile
		Sporulation	non-sporulating
		Temperature range	mesophilic
		Optimum temperature	not reported
	Salinity	tolerates up to 15g NaCl/L	TAS [1]
	Oxygen requirement	essentially aerobic	
		glucose and sucrose	
		Carbon source	no acid production from glucose detectable aerobically, carbohydrates such as sugars, sugar alcohols and carbonic acids but no polymers such as starch, cellulose or gelatin
	Energy source	ferments glucose and sucrose	TAS [1]
		endophytic in stems of maize plants	TAS [2]
		cysts of nematode <i>Heterodera glycines</i>	TAS [3]
MIGS-6	Habitat	contaminated soil	TAS [4]
MIGS-15	Biotic relationship	free-living	
MIGS-14	Pathogenicity	none	TAS [1]
	Biosafety level	1	TAS [18]
	Isolation	surface sterilized stems of maize plants	
		grown in sterile soil without nitrogen fertilizer under green house conditions	TAS [1]
MIGS-4	Geographic location	Madison-Wisconsin; USA	
MIGS-5	Sample collection time	not reported	
MIGS-4.1	Latitude – Longitude	43.1, -89.4	
MIGS-4.2			
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from <http://www.geneontology.org/GO.evidence.shtml> of the Gene Ontology project [19]. If the evidence code is IDA, then the property should have been directly observed, for the purpose of this specific publication, for a live isolate by one of the authors, or an expert or reputable institution mentioned in the acknowledgements.

## Genome sequencing and annotation information

### Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position, and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project. The genome project is deposited in the Genomes OnLine Database [14] and the complete genome sequence in GenBank NOT YET. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

**Table 2.** Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	Two genomic Sanger libraries: 8 kb pMCL200 and fosmid pcc1Fos
MIGS-29	Sequencing platforms	ABI3730
MIGS-31.2	Sequencing coverage	10.6x Sanger
MIGS-30	Assemblers	Phred/Phrap/Consed
MIGS-32	Gene calling method	Prodigal
	INSDC / Genbank ID	N/A
	Genbank Date of Release	N/A
	GOLD ID	<a href="#">Gc01069</a>
	Database: IMG-GEBA	<a href="#">2501416930</a>
	NCBI project ID	<a href="#">20829</a>
MIGS-13	Source material identifier	DSM <a href="#">18053</a>
	Project relevance	Tree of Life, GEBA

### Growth conditions and DNA isolation

*D. fermentans* NS114<sup>T</sup>, DSM18053, was grown in DSMZ medium 830 (R2A Medium; see [http://www.dsmz.de/microorganisms/media\\_list.php](http://www.dsmz.de/microorganisms/media_list.php)) at 28°C. DNA was isolated from 1-1.5 g of cell paste using Qiagen Genomic 500 DNA Kit (Qiagen, Hilden, Germany) with a modified protocol for cell lysis including 20 min. freezing (-70°C), 5 min. heating (98°C), 15 min. cooling to 37°C; adding 1.5 ml lysozyme (standard: 0.3 ml, only), 1.0 ml achromopeptidase, 0.12 ml lysostaphine, 0.12 ml mutanolysine, 1.5 ml proteinase K (standard: 0.5 ml, only), and overnight incubation at 35°C on a shaker.

### Genome sequencing and assembly

The genome was sequenced using a combination of 8 kb and fosmid DNA libraries. All general aspects of library construction and sequencing performed at the JGI can be found at

<http://www.jgi.doe.gov/>. Draft assemblies were based on 86,260 total reads. The Phred/Phrap/Consed software package (<http://www.phrap.com>) was used for sequence assembly and quality assessment [20-22]. After the shotgun stage, reads were assembled with parallel phrap. Possible mis-assemblies were corrected with Dupfinisher or transposon bombing of bridging clones [23]. Gaps between contigs were closed by editing in Consed, custom primer walk or PCR amplification. A total of 1042 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. The error rate of the completed genome sequence is less than 1 in 100,000. Together all libraries provided 10.6x coverage of the genome.

### Genome annotation

Genes were identified using Prodigal [24] as part of the genome annotation pipeline in the Integrated Microbial Genomes Expert Review (IMG-ER) system (<http://img.jgi.doe.gov/er>) [25], followed by a round of manual curation using the JGI GenePRIMP pipeline (<http://geneprimp.jgi-psf.org>). The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes Expert Review (IMG-ER) platform (<http://img.jgi.doe.gov/>) [26].

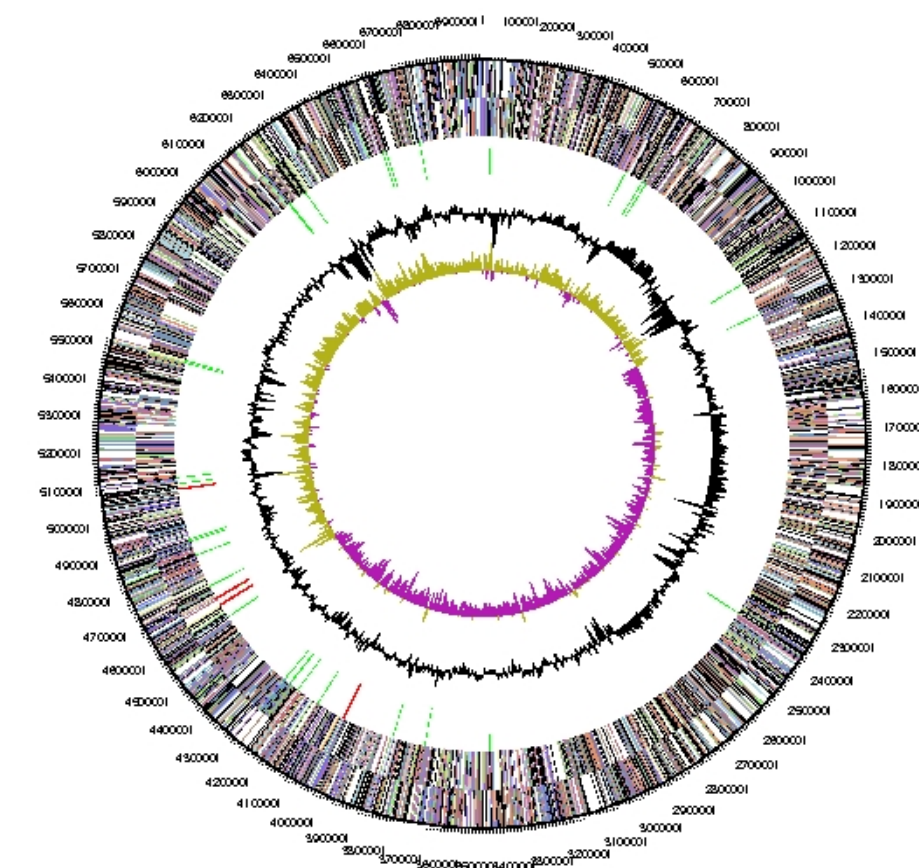
### Genome properties

The genome is 6,967,790 bp long and comprises one main circular chromosome with a 51.4% GC content (Table 3, Figure 3). Of the 5854 genes predicted, 5804 were protein coding genes, and 50 were RNAs. Eighty-five pseudogenes were also identified. The majority of the protein-coding genes (64.7%) were assigned with a putative function while the remaining were annotated as hypothetical proteins. The properties and the statistics of the genome are summarized in Table 3. The distribution of genes into COGs functional categories is presented in Table 4.

**Table 3.** Genome Statistics

Attribute	Value	% of Total
Genome size (bp)	6,967,790	100.00%
DNA Coding region (bp)	6,343,890	91.05%
DNA G+C content (bp)	3,591,547	51.54%
Number of replicons	1	
Extrachromosomal elements	0	
Total genes	5854	100.00%
RNA genes	50	0.85%
rRNA operons	4	
Protein-coding genes	5804	99.15%
Pseudo genes	85	1.45%
Genes with function prediction	3790	64.74%
Genes in paralog clusters	1250	21.35%
Genes assigned to COGs	3702	63.24%
Genes assigned Pfam domains	3853	65.82%
Genes with signal peptides	1760	30.06%
Genes with transmembrane helices	1239	21.17%
CRISPR repeats	0	





**Figure 3. Graphical circular map of the genome.** From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

**Table 4.** Number of genes associated with the 21 general COG functional categories

Code	COG counts and percentage of protein-coding genes		Description
Genome			
	value	% of total	
J	171	2.9	Translation
A	0	0.0	RNA processing and modification
K	392	6.8	Transcription
L	157	2.7	Replication, recombination and repair
B	0	0.0	Chromatin structure and dynamics
D	23	0.4	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	111	1.9	Defense mechanisms
T	343	5.9	Signal transduction mechanisms
M	345	5.9	Cell wall/membrane biogenesis
N	13	0.2	Cell motility
Z	1	0.0	Cytoskeleton

W	0	0.0	Extracellular structures
U	56	1.0	Intracellular trafficking and secretion
O	110	1.9	Posttranslational modification, protein turnover, chaperones
C	179	3.1	Energy production and conversion
G	335	5.8	Carbohydrate transport and metabolism
E	247	4.4	Amino acid transport and metabolism
F	75	1.3	Nucleotide transport and metabolism
H	190	3.3	Coenzyme transport and metabolism
I	143	2.5	Lipid transport and metabolism
P	295	5.1	Inorganic ion transport and metabolism
Q	102	1.8	Secondary metabolites biosynthesis, transport and catabolism
R	540	9.3	General function prediction only
S	345	5.9	Function unknown
-	2102	36.2	Not in COGs

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